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The immunological consequences of photodynamic treatment of cancer, a literature review

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Abstract

In this review we discuss the effect of photodynamic treatment (PDT) of solid tumors on the immune response. The effect on both the innate and adapted immune response is discussed. We have summarized the evidence that PDT causes or enhances an anti-tumor response. PDT is a local treatment in which the treated tumor remains *in situ* while the immune system is only locally affected and still functional in contrast with e.g. after systemic chemotherapy. We conclude that PDT of cancer is a way of *in situ* vaccination to induce a systemic anti-tumor response. In general, immune cells are found in the tumor stroma, separated from tumor cells by extracellular matrix and basal membrane-like structures. We hypothesize that PDT destroys the structure of a tumor, thereby enabling direct interaction between immune cells and tumor cells resulting in the systemic anti-tumor immune response.

Abbreviations: ALA = 5-aminolaevulinic acid; APC = antigen presenting cells; CHS = contact hypersensitivity response; DBPMAF = vitamin D_3-binding protein derived macrophage-activating factor; G-CSF = granulocyte colony stimulating factor; HpD = haematoporphyrin derivative; PDT = photodynamic therapy; PIT = photoimmunotherapy

Introduction

Tumor cells and the nourishing microvasculature are the targets of photodynamic therapy (PDT), resulting in primary tumor cell death via a direct and indirect pathway. Direct tumor cell death occurs when during light irradiation, energy is transferred from the excited photosensitizer onto oxygen molecules, generating the formation of singlet oxygen (type II photochemical reaction). This highly reactive molecule causes direct photodamage of proteins, lipids and other molecules at the sites where the photosensitizer accumulates, leading to PDT-mediated direct tumor cell killing either by apoptosis or necrosis. The mode of cell death upon PDT depends on several factors. The intracellular localization of the photosensitizer is of great importance, since damage to mitochondria generally leads to apoptosis, whereas plasma membrane damage can delay or even inhibit apoptosis and instead induces necrosis (Kessel et al., 1997; Luo & Kessel, 1997; Kessel & Luo, 1998; Chen et al., 2000; Fabris et al., 2001). Haematoporphyrin derivative (HpD) and its purified form Photofrin, one of the most widely used

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photosensitizers, localize in mitochondria due to both their hydrophobicity and the affinity for a plasma binding site on the mitochondrial membrane that also binds benzodiazepines (Roberts & Burns, 1989; Shulok et al., 1990; Wilson et al., 1997). Another often-used drug in PDT is 5-aminolaevulinic acid (ALA). This is not a photosensitizer, but a precursor of the endogenous photosensitizer protoporphyrin IX in the haem synthesis pathway, which is produced in the mitochondria (Liang et al., 1998; Chen et al., 2001). Other more hydrophilic sensitizers such as phthalocyanines and many chlorines enter the cells via endocytosis and hence accumulate mainly in lysosomes. When cells are illuminated, relocalization occurs throughout the cytosol (Wood et al., 1997; Ball et al., 1999; Pogue et al., 2001). Also the dose of photosensitizer and/or light influences the occurrence of either necrosis or apoptosis. For apoptosis the presence of functional enzymes is necessary, therefore if PDT dose is too high, apoptosis cannot occur. This is confirmed by several researchers who showed that low dose *in vitro* PDT results in apoptosis whereas high dose PDT leads to necrosis (Oleinick & Evans, 1998; Lavie et al., 1999; Di Stefano et al., 2001; Vantieghem et al., 2001). Studies with apoptosis inhibitors and with cell lines that lack functional caspase-3 show that when the apoptotic pathway is blocked, necrosis occurs instead so the overall effect of treatment is still the same (Wyld et al., 2001; Thibaut et al., 2002). However, there may be differences in the occurrence of a systemic immune response, since necrosis more than apoptosis leads to inflammation, which is the first step in induction of a specific immune response.

For complete eradication and long-term control of tumors, indirect tumor cell killing seems as important as direct cellular damage. Phototoxic lesions in the endothelium of tumor blood vessels lead to severe and persistent post-PDT tumor hypoxia/anoxia (Star et al., 1986). These vascular effects of PDT are caused by reversible contraction of endothelial cells resulting in the exposure of the basement membrane, macromolecular vessel leakage, leukocyte adhesion and thrombus formation (Nelson et al., 1988; Fingar et al., 1992). All are apparently linked to platelet activation and the release of thromboxane (Fingar et al., 1990, 1993). PDT may also inhibit the production or release of nitric oxide by the endothelium, leading to more vessel constriction (Gilissen et al., 1993). This chain of events leads to complete vascular occlusion and the ischemic

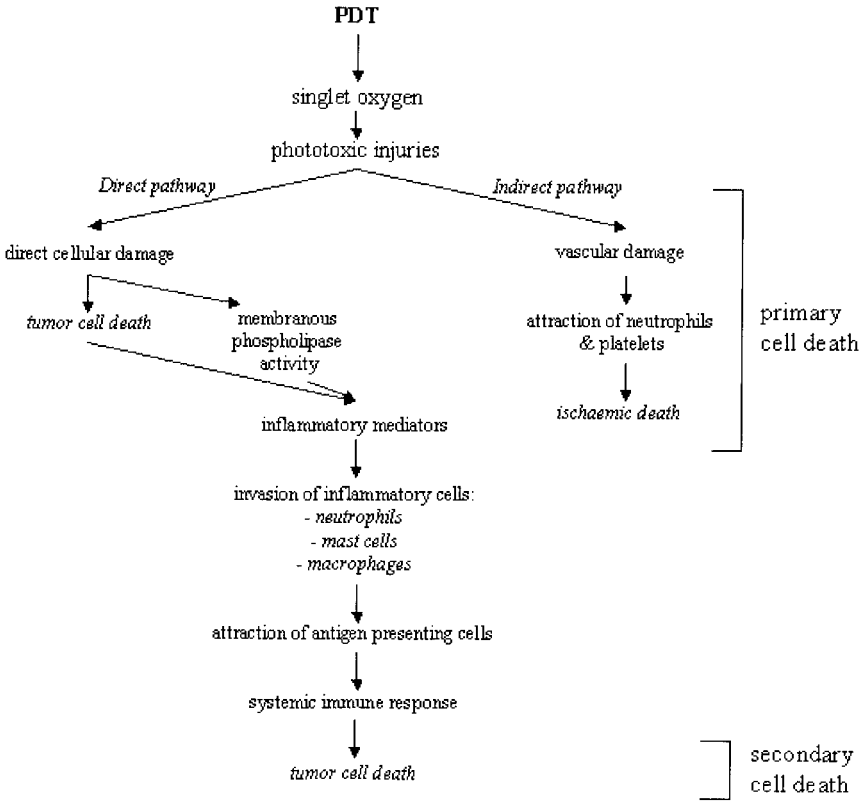

Fig. 1. Schematic representation of direct and indirect courses of tumor destruction upon PDT, as explained in detail in the text.

death of remaining tumor cells. A schematic representation of how tumors are destroyed by PDT is shown in figure 1.

Induction of an Anti-tumor Immune Response by PDT

After this primary tumor cell death, there may be a secondary cause of tumor cell death, mediated by a systemic anti-tumor immune response induced after PDT. Animal models have shown that this may be an additional effect of PDT of cancer.

PDT of EMT6 mammary sarcoma in SCID and normal mice showed a significantly lower therapeutic effect in SCID mice, suggesting that the difference in tumor cures originated in the lack of an immune response (Korbelik et al., 1996). This effect could be restored by adoptive transfer of T-lymphocytes of normal (BALB/c) mice into the SCID mice. Hendrzak-Henion and colleagues (1999) found similar results in a study with T cell deficient mice. In a later study, Korbelik and Celic (1999) selectively depleted EMT6 sarcoma-bearing mice of specific myeloid (neutrophils, macrophages) and lymphoid (T cells) populations. Although immunodepletion of T cells did not affect the initial tumor ablation by PDT, the tumor cure rate was significantly reduced, showing the importance of the immunological contribution to the effect of PDT.

The hypothesis is also supported by findings of regression of untreated metastases at a distant site from the treated primary tumors (Chen et al., 1999). Accordingly, tumor rechallenge in rats effectively treated with PDT did not lead to tumor growth suggesting that an immunological memory had been generated by the initial PDT treatment. This tumor immunity appeared to be transferable (Korbelik et al., 1996).

These studies confirm the existence of a systemic anti-tumor response after PDT, in which T cells play a crucial role.

How Does PDT Affect the Immune System?

Direct effect of PDT on immune cells

The direct effect of PDT on immune cells may be harmful. Lymphocytes, especially activated T cells, accumulate photosensitizer and can therefore be killed by subsequent illumination, leading to a local suppression of the immune system (Hryhenko et al., 1998; Hunt et al., 1999; Casas et al., 2002).

One of the parameters to measure this effect of PDT on immune cells is a contact hypersensitivity response (CHS), a classical example of a cell-mediated delayed-type immune reaction with epidermal Langerhans cells, macrophages, and T-lymphocytes participating. In the 1980's Elmets and Bowen (1986) already described the suppression of this response upon PDT treatment of the skin. A study by Gollnick et al. (1997) indicated that an increase in IL-10 might be responsible for this immunosuppressive effect of PDT, but a later study by the same author showed that suppression of CHS upon cutaneous PDT did not occur via an IL-10 dependent mechanism (Gollnick et al., 2001a). Transcutaneous whole body PDT however was shown to be IL-10 dependent by Simkin et al. (2000). An adoptive transfer study in mice by Jolles et al. (1988) showed that the reduction of the CHS also extended to areas other than the illuminated site. This phenomenon was associated with the generation of suppressor cells and an acute-phase response, characterized by leucocytosis and elevated serum amyloid P levels. In a study by Qin et al. (1993) allograft survival was prolonged after pretreatment of the peritoneum with HpD-PDT. Nearly complete depletion of peritoneal lymphocytes was observed in mice after PDT treatment of the exposed musculoperitoneal layer, but also a loss of responsiveness of splenic lymphocytes to mitogens was found. *In vitro* PDT of macrophages had a similar harmful effect, leading to decreased viability (Steubing et al., 1991; Reiter et al., 1999).

In another study on allograft survival and the immunosuppressive effects of PDT, Obochi and co-workers (1997) propose that low-dose PDT of donor skin tissue induces a series of down regulatory events in the graft, resulting in decreased expression of MHC and costimulatory molecules on the surface of donor Langerhans cells. King et al. (1999) found a similar reduction in expression of MHC antigens and costimulatory molecules such as CD80 and CD86 on mouse dendritic cells after treatment with PDT. As a result of this decreased expression of surface MHC class II molecules, Langerhans cells largely lose the ability to stimulate the efficient proliferation of allogenic T-cells. This impairment of antigen presenting capacity was also demonstrated by Hryhorenko and co-workers (1998) who showed in an *in vitro* study with human peripheral blood monocytes in a mixed leucocyte reaction that the ability of antigen presenting cells (APC) to properly present antigens to T cells significantly decreases

after treatment of APC with PDT. Similar findings were published by Barret and Gruner, with protoporphyrin and HpD-based PDT respectively (Gruner et al., 1986; Barrett et al., 1994). These studies thus show the antigen presenting capacity of monocytes and dendritic cells to decrease after PDT, resulting in decreased contact hypersensitivity response and increased allograft survival.

Systemic effects on the immune system upon local PDT tumor treatment

In general, immune cells are found in the tumor stroma, separated from tumor cells by extracellular matrix and basal membrane-like structures i.e. there is no or minimal direct interaction (Hagenaars et al., 2000; Kuppen et al., 2001). It is conceivable that PDT destroys the structure of a tumor, thereby enabling direct interaction between immune cells and tumor cells. After the initial PDT-induced damage to tumor cells and immune cells, indeed a strong inflammatory reaction occurs locally, which leads to influx and activation of undamaged immune cells from elsewhere.

Photodamage of membrane lipids results in the activation of membranous phospholipases, leading to a massive release of lipid fragments and metabolites of arachidonic acid (Henderson & Donovan, 1989; Agarwal et al., 1993). Both are potent inflammatory mediators. Furthermore, changes in the blood vessel walls, as described above (e.g. exposure of the basement membrane), will attract neutrophils and platelets (de Vree et al., 1996a). These cells will also release more inflammatory mediators, which chemotactic capacities enable a massive recruitment of immune cells to the damaged site. PDT is associated with elevated expression and/or production of several cytokines: IL-1β, IL-2, IL-6, IL-10, TNF-α and granulocyte colony-simulating factor (G-CSF) (Herman et al., 1996; Gollnick et al., 1997; Ziolkowski et al., 1997; Chen et al., 2000; Blank et al., 2001; Gollnick et al., 2001b; Usuda et al., 2001). These cytokines are important messenger proteins, regulating the inflammatory and immunological responses towards bacteria, viruses and tumors. Release of these chemotactic factors from affected tissue results in the attraction and accumulation of non-lymphoid inflammation cells. Within minutes after PDT treatment a large number of neutrophils invade the area (Krosl et al., 1995; Gollnick et al., 1997). Most likely, these phagocytes are present at the inflammatory site to remove cell debris caused by PDT. Neutrophils appear to be at least partially dependent on an IL-1-induced release of granulocyte colony stimulating factor (G-CSF) (de Vree et al., 1997). De Vree et al. (1996b) found that PDT of rhabdomyosarcoma-bearing rats resulted in neutrophilia and an increase in IL-1 serum levels. Administration of anti-G-CSF antibody led to a decrease in PDT efficacy. Although elevated neutrophil levels were also found in control rats, which received surgery only, a delay in tumor growth was not detected here. It was concluded that PDT with the help of IL-1 and G-CSF had a stimulatory effect on neutrophil activity, turning them into direct tumor cell killers. The importance of the role of neutrophils in the anti-tumor effect of PDT was also shown by depletion of neutrophils in mice directly after PDT treatment of subcutaneous EMT6 mammary sarcoma, which resulted in 70% reduction of the curative effect, as measured by the percentage of tumor-free mice after 90 days. Depletion of neutrophils did not affect the initial tumor ablation, induced within the 24 hours after PDT (Korbelic & Cecic, 1999).

Neutrophil invasion is followed by the arrival of mast cells, lymphocytes, monocytes and macrophages (Krosl et al., 1995). Although direct in vitro PDT of macrophages results in decreased viability of these cells (Steubing et al., 1991; Reiter et al., 1999), several researchers described an increase in phagocytic capacities when low drug and light doses are administered in both *in vitro* and *in vivo* studies (Yamamoto et al., 1994; Coutier et al., 1999). A point of discussion is whether PDT activates macrophages directly, or indirectly via increased susceptibility of tumor cells to phagocytosis. Reiter et al. (1997) found support for the indirect activation of macrophages since in their studies, HpD-PDT treated macrophages did not exhibit increased cytotoxicity against YAC-1 murine T-lymphoma cells whereas photodynamic treatment of tumor cells did result in activation of tumoricidal effector functions of macrophages. A later study showed that when PDT-treated carcinoma cells were added *in vitro* to macrophages, these macrophages became more cytotoxic to other carcinoma cells, which fortified the hypothesis that macrophages are stimulated by damaged tumor cells rather than by PDT (Reiter et al., 1999). Yamamoto et al. (1991) however found that *in vitro* PDT of macrophages only led to higher ingestion activity when B- and T-lymphocytes were present together with the macrophages during PDT, suggesting a mechanism whereby damage to cell membranes of lymphocytes leads to activation of macrophages. In two other studies, macrophages were harvested from PDT-treated tumors and from the PDT-treated peritoneum. These macrophages exhibit increased cytolytic capacities, but since both lymphocytes and tumor cells were

present in this *in vivo* situation, these studies seemingly can not sufficiently refute the aforementioned results (Qin et al. ,1993; Krosl et al., 1995). As macrophage inactivation with silica particles injected immediately post-PDT intra-peritoneally in mice with EMT6 sarcoma resulted in 40% less tumor-free mice after 90 days, it can be assumed that their role in PDT efficacy is considerable (Korbelik & Cecic, 1999).

After phagocytosis and processing of tumor cell debris, macrophages may function as APC (Ziegler & Unanue, 1981). In contrast to e.g. radiotherapy, PDT induces a rapid release of tumor cell debris that may enhance the uptake and presentation of tumor antigens by tumor-associated APC and thus lead to lymphocyte involvement, as shown by infiltration of vulval intraepithelial neoplasia with cytotoxic T lymphocytes after treatment with ALA-based PDT (Abdel-Hady et al., 2001). The ensuing systemic immune response may result in destruction of locally remaining tumor cells as well as prevent the occurrence of distant metastases. This is adequately shown by animal studies in which treatment of the primary tumor with PDT resulted in a decrease in distant metastases when compared to animals of which the tumours were resected or not treated at all (Gomer et al., 1987; van Hillegersberg et al., 1995; Schreiber et al., 2002). Chen et al. (1999) showed this anti tumor effect not only on newly formed metastases but also on established metastases in a rat model, although it must be noted that in this study the immunoadjuvant glycated chitosan was linked to the photosensitizer. Rats were implanted in this study with metastatic mammary tumor cells in inguinal fat pads, resulting in primary tumors after 5 days that metastasised to remote inguinal and axillary regions after 15–20 days. After successful PDT treatment of the primary tumors, rats were rechallenged 120 days after initial inoculation with 10^6 tumor cells. PDT treatment resulted in disappearance of the metastases of the primary tumor as well as in total resistance to rechallenge. Accordingly, Korbelik et al. (1996) found that tumor rechallenge in rats effectively treated with PDT did not lead to tumor growth suggesting that an immunological memory had been generated by the initial PDT treatment. This tumor immunity appeared to be transferable. These studies strongly suggest the induction of a systemic anti-tumor immune response after PDT. Since local PDT treatment enhances or causes this systemic immune response, it could be regarded as a way of *in situ* vaccination. An important factor in this may be that PDT causes the destruction of extracellular matrix that surrounds established tumor nodules and thus enables interaction between immune cells and tumor cells (Hagenaars et al., 2000).

Photoimmunotherapy

The developing field of photoimmunotherapy (PIT), a combination of PDT and immunotherapy, deserves special interest. This combination aims at potentiating the cytotoxic effect of PDT by improvement of the tumor specific localisation of the photosensitizer and/or by boosting specific parts of the immune system.

Selective treatment of tumors may be achieved by binding of the photosensitizer to a tumor-specific antibody. Del Governatore et al. (2000) performed an *in vivo* study with a chlorin-e6/Mab 17.1A conjugate, directed against the antigen EpCAM that is associated with carcinomas of epithelial origin. Tumor reduction and median survival were significantly higher in the PIT treated rats when compared to immunotherapy or PDT alone.

PIT may also consist of PDT combined with a specific immuno-enhancer such as granulocyte-macrophage colony stimulating factor (GM-CSF) (de Vree et al., 1997). This cytokine controls the maturation and function of granulocytes and macrophages, and also stimulates the proliferation and differentiation of dendritic cells and other antigen-presenting cells. Reports by Golab et al. (2000) and Krosl et al. (1996) both demonstrate a substantially improved curative anti-tumor effect of PDT when tumor-localized GM-CSF immuno-adjuvant treatment was administered. The same effects were found when vitamin D_3-binding protein-derived macrophage-activating factor (DBPMAF) (Korbelik et al., 1997) and glucan schizophyllan (Krosl & Korbelik, 1994) were used *in vitro*. It is suggested that PDT is highly receptive to immuno-adjuvant therapy with a macrophage-activating factor, thus fortifying the hypothesis that the immune system plays an important role in the long-term tumor eradication after PDT.

Immune potentiation can also be found when applying PDT *in vitro* to a transfected IL-6 producing Lewis lung carcinoma cell line, which led to increased sensitivity of cells to PDT as measured by MTT assay. A combination of PDT and IL-6 administration may therefore be able to enhance the anti-tumor effect of PDT (Usuda et al., 2001). Korbelik and co-workers (2001) showed higher cure rates for PDT of human cervical squamous cell carcinoma and human colorectal adenocarcinoma in mice when combining PDT with adoptive transfer

of IL-2 producing NK-cells. Transfected IL-2 producing NK cells were administered peritumoral or intravenously immediately after PDT, resulting in improved cure rates when compared to PDT treatment without NK-cells or with non-IL-2 producing NK cells.

Conclusion

As PDT locally damages the tumor structure, it enables influx of non-affected immune cells into the PDT site, resulting in the development of a systemic anti-tumor immune response. The immune system should therefore be protected from harmful effects of PDT, for example by applying tumor treatment locally only, preferably in combination with immune enhancing treatment, e.g. by using systemically applied cytokines or immune modulators as GM-CSF, G-CSF, DPBMAF and glucan schizophyllan. However, clinical trials are necessary to assess the applicability of these experimental *in vivo* and *in vitro* combination therapies. Future research must be focussed on PDT as a way of *in situ* vaccination in cancer therapy to cure and prevent the formation of distant metastases.

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References

Abdel-Hady ES, Martin-Hirsch P, Duggan-Keen M, Stern PL, Moore JV, Corbitt G, Kitchener HC and Hampson IN. 2001. Immunological and viral factors associated with the response of vulval intraepithelial neoplasia to photodynamic therapy. Cancer Res. 61: 192–196.

Agarwal ML, Larkin HE, Zaidi SI, Mukhtar H and Oleinick NL. 1993. Phospholipase activation triggers apoptosis in photosensitized mouse lymphoma cells. Cancer Res. 53: 5897–5902.

Ball DJ, Mayhew S, Wood SR, Griffiths J, Vernon DI and Brown SB. 1999. A comparative study of the cellular uptake and photodynamic efficacy of three novel zinc phthalocyanines of differing charge. Photochem. Photobiol. 69: 390–396.

Barrett KE, Yen A, Bigby TD, Montisano D and Gigli I. 1994. Inhibition of human peripheral blood lymphocyte function by protoporphyrin and longwave ultraviolet light. J. Immunol. 153: 3286–3294.

Blank M, Lavie G, Mandel M and Keisari Y. 2001. Effects of photodynamic therapy with hypericin in mice bearing highly invasive solid tumors. Oncol. Res. 12: 409–418.

Casas A, Perotti C, Fukuda H and Batlle AMdC. 2002. Photodynamic therapy of activated and resting lymphocytes and its antioxidant adaptive response. Lasers Med. Sci. 17: 42–50.

Chen WR, Zhu WG, Dynlacht JR, Liu H and Nordquist RE. 1999. Long-term tumor resistance induced by laser photo-immunotherapy. Int. J. Cancer 81: 808–812.

Chen JY, Cheung NH, Fung MC, Wen JM, Leung WN and Mak NK. 2000. Subcellular localization of merocyanine 540 (MC540) and induction of apoptosis in murine myeloid leukemia cells. Photochem. Photobiol. 72: 114–120.

Chen JY, Mak NQ, Cheung NH, Leung RN and Peng Q. 2001. Endogenous production of protoporphyrin IX induced by 5-aminolevulinic acid in leukemia cells. Acta Pharmacol. Sin. 22: 163–168.

Coutier S, Bezdetnaya L, Marchal S, Melnikova V, Belitchenko I, Merlin JL and Guillemin F. 1999. Foscan (mTHPC) photosensitized macrophage activation: enhancement of phagocytosis, nitric oxide release and tumour necrosis factor-alpha-mediated cytolytic activity. Br. J. Cancer 81: 37–42.

Del Governatore M, Hamblin MR, Shea CR, Rizvi I, Molpus KG, Tanabe KK and Hasan T. 2000. Experimental photoimmunotherapy of hepatic metastases of colorectal cancer with a 17.1A chlorin(e6) immunoconjugate. Cancer Res. 60: 4200–4205.

de Vree WJ, Fontijne-Dorsman AN, Koster JF and Sluiter W. 1996a. Photodynamic treatment of human endothelial cells promotes the adherence of neutrophils in vitro. Br. J. Cancer 73: 1335–1340.

de Vree WJ, Essers MC, de Bruijn HS, Star WM, Koster JF and Sluiter W. 1996b. Evidence for an important role of neutrophils in the efficacy of photodynamic therapy in vivo. Cancer Res. 56: 2908–2911.

de Vree WJ, Essers MC, Koster JF and Sluiter W. 1997. Role of interleukin 1 and granulocyte colony-stimulating factor in photofrin-based photodynamic therapy of rat rhabdomyosarcoma tumors. Cancer Res. 57: 2555–2558.

Di Stefano A, Ettorre A, Sbrana S, Giovani C and Neri P. 2001. Purpurin-18 in combination with light leads to apoptosis or necrosis in HL60 leukemia cells. Photochem. Photobiol. 73: 290–296.

Elmets CA and Bowen KD. 1986. Immunological suppression in mice treated with hematoporphyrin derivative photoradiation. Cancer Res. 46: 1608–1611.

Fabris C, Valduga G, Miotto G, Borsetto L, Jori G, Garbisa S and Reddi E. 2001. Photosensitization with zinc (II) phthalocyanine as a switch in the decision between apoptosis and necrosis. Cancer Res. 61: 7495–7500.

Fingar VH, Wieman TJ and Doak KW. 1990. Role of thromboxane and prostacyclin release on photodynamic therapy- induced tumor destruction. Cancer Res. 50: 2599–2603.

Fingar VH, Wieman TJ, Wiehle SA and Cerrito PB. 1992. The role of microvascular damage in photo-

dynamic therapy: the effect of treatment on vessel constriction, permeability, and leukocyte adhesion. Cancer Res. 52: 4914–4921.

Fingar VH, Siegel KA, Wieman TJ and Doak KW. 1993. The effects of thromboxane inhibitors on the microvascular and tumor response to photodynamic therapy. Photochem. Photobiol. 58: 393–399.

Gilissen MJ, Merbel-de Wit LE, Star WM, Koster JF and Sluiter W. 1993. Effect of photodynamic therapy on the endothelium-dependent relaxation of isolated rat aortas. Cancer Res. 53: 2548–2552.

Golab J, Wilczynski G, Zagozdzon R, Stoklosa T, Dabrowska A, Rybczynska J, Wasik M, Machaj E, Olda T, Kozar K, Kaminski R, Giermasz A, Czajka A, Lasek W, Feleszko W and Jakobisiak M. 2000. Potentiation of the anti-tumour effects of Photofrin-based photodynamic therapy by localized treatment with G-CSF. Br. J. Cancer 82: 1485–1491.

Gollnick SO, Liu X, Owczarczak B, Musser DA and Henderson BW. 1997. Altered expression of interleukin 6 and interleukin 10 as a result of photodynamic therapy in vivo. Cancer Res. 57: 3904–3909.

Gollnick SO, Musser DA, Oseroff AR, Vaughan L, Owczarczak B and Henderson BW. 2001a. IL-10 does not play a role in cutaneous Photofrin photodynamic therapy-induced suppression of the contact hypersensitivity response. Photochem. Photobiol. 74: 811–816.

Gollnick SO, Lee BY, Vaughan L, Owczarczak B and Henderson BW. 2001b. Activation of the IL-10 gene promoter following photodynamic therapy of murine keratinocytes. Photochem. Photobiol. 73: 170–177.

Gomer CJ, Ferrario A and Murphree AL. 1987. The effect of localized porphyrin photodynamic therapy on the induction of tumour metastasis. Br. J. Cancer 56: 27–32.

Gruner S, Volk HD, Noack F, Meffert H and von Baehr R. 1986. Inhibition of HLA-DR antigen expression and of the allogeneic mixed leukocyte reaction by photochemical treatment. Tissue Antigens 27: 147–154.

Hagenaars M, Ensink NG, Basse PH, Hokland M, Nannmark U, Eggermont AM, van de Velde CJ, Fleuren GJ and Kuppen PJ. 2000. The microscopic anatomy of experimental rat CC531 colon tumour metastases: consequences for immunotherapy? Clin. Exp. Metastasis 18: 189–196.

Henderson BW and Donovan JM. 1989. Release of prostaglandin E2 from cells by photodynamic treatment in vitro. Cancer Res. 49: 6896–6900.

Hendrzak-Henion JA, Knisely TL, Cincotta L, Cincotta E and Cincotta AH. 1999. Role of the immune system in mediating the antitumor effect of benzophenothiazine photodynamic therapy. Photochem. Photobiol. 69: 575–581.

Herman S, Kalechman Y, Gafter U, Sredni B and Malik Z. 1996. Photofrin II induces cytokine secretion by mouse spleen cells and human peripheral mononuclear cells. Immunopharmacology 31: 195–204.

Hryhorenko EA, Oseroff AR, Morgan J and Rittenhouse-Diakun K. 1998. Antigen specific and non-specific modulation of the immune response by aminolevulinic acid based photodynamic therapy. Immunopharmacology 40: 231–240.

Hunt DW, Jiang H, Granville DJ, Chan AH, Leong S and Levy JG. 1999. Consequences of the photodynamic treatment of resting and activated peripheral T lymphocytes. Immunopharmacology 41: 31–44.

Jolles CJ, Ott MJ, Straight RC and Lynch DH. 1988. Systemic immunosuppression induced by peritoneal photodynamic therapy. Am. J. Obstet. Gynecol. 158: 1446–1453.

Kessel D and Luo Y. 1998. Mitochondrial photodamage and PDT-induced apoptosis. J. Photochem. Photobiol. B 42: 89–95.

Kessel D, Luo Y, Deng Y and Chang CK. 1997. The role of subcellular localization in initiation of apoptosis by photodynamic therapy. Photochem. Photobiol. 65: 422–426.

King DE, Jiang H, Simkin GO, Obochi MO, Levy JG and Hunt DW. 1999. Photodynamic alteration of the surface receptor expression pattern of murine splenic dendritic cells. Scand. J. Immunol. 49: 184–192.

Korbelik M and Cecic I. 1999. Contribution of myeloid and lymphoid host cells to the curative outcome of mouse sarcoma treatment by photodynamic therapy. Cancer Lett. 137: 91–98.

Korbelik M, Krosl G, Krosl J and Dougherty GJ. 1996. The role of host lymphoid populations in the response of mouse EMT6 tumor to photodynamic therapy. Cancer Res. 56: 5647–5652.

Korbelik M, Naraparaju VR and Yamamoto N. 1997. Macrophage-directed immunotherapy as adjuvant to photodynamic therapy of cancer. Br. J. Cancer 75: 202–207.

Korbelik M, Sun J and Posakony JJ. 2001. Interaction between photodynamic therapy and BCG immunotherapy responsible for the reduced recurrence of treated mouse tumors. Photochem. Photobiol. 73: 403–409.

Krosl G and Korbelik M. 1994. Potentiation of photodynamic therapy by immunotherapy: the effect of schizophyllan (SPG). Cancer Lett. 84: 43–49.

Krosl G, Korbelik M and Dougherty GJ. 1995. Induction of immune cell infiltration into murine SCCVII tumour by photofrin-based photodynamic therapy. Br. J. Cancer 71: 549–555.

Krosl G, Korbelik M, Krosl J and Dougherty GJ. 1996. Potentiation of photodynamic therapy-elicited anti-tumor response by localized treatment with granulocyte-macrophage colony-stimulating factor. Cancer Res. 56: 3281–3286.

Kuppen PJ, van der Eb MM, Jonges LE, Hagenaars M, Hokland ME, Nannmark U, Goldfarb RH, Basse PH, Fleuren GJ, Hoeben RC and van de Velde CJ. 2001. Tumor structure and extracellular matrix as a possible barrier for therapeutic approaches using immune cells or adenoviruses in colorectal cancer. Histochem. Cell Biol. 115: 67–72.

Lavie G, Kaplinsky C, Toren A, Aizman I, Meruelo D, Mazur Y and Mandel M. 1999. A photodynamic pathway to apoptosis and necrosis induced by dimethyl tetrahydroxyhelianthrone and hypericin in leukaemic cells: possible relevance to photodynamic therapy. Br. J. Cancer 79: 423–432.

Liang H, Shin DS, Lee YE, Nguyen DC, Trang TC, Pan AH, Huang SL, Chong DH and Berns MW. 1998. Subcellular phototoxicity of 5-aminolaevulinic acid (ALA). Lasers Surg. Med. 22: 14–24.

Luo Y and Kessel D. 1997. Initiation of apoptosis versus necrosis by photodynamic therapy with chloroaluminum phthalocyanine. Photochem. Photobiol. 66: 479–483.

Nelson JS, Liaw LH, Orenstein A, Roberts WG and Berns MW. 1988. Mechanism of tumor destruction following photodynamic therapy with hematoporphyrin derivative, chlorin, and phthalocyanine. J. Natl. Cancer Inst. 80: 1599–1605.

Obochi MO, Ratkay LG and Levy JG. 1997. Prolonged skin allograft survival after photodynamic therapy associated with modification of donor skin antigenicity. Transplantation 63: 810–817.

Oleinick NL and Evans HH. 1998. The photobiology of photodynamic therapy: cellular targets and mechanisms. Radiat. Res. 150: S146–S156.

Pogue BW, Ortel B, Chen N, Redmond RW and Hasan T. 2001. A photobiological and photophysical-based study of phototoxicity of two chlorins. Cancer Res. 61: 717–724.

Qin B, Selman SH, Payne KM, Keck RW and Metzger DW. 1993. Enhanced skin allograft survival after photodynamic therapy. Association with lymphocyte inactivation and macrophage stimulation. Transplantation 56: 1481–1486.

Reiter I, Schwamberger G and Krammer B. 1997. Effect of photodynamic pretreatment on the susceptibility of murine tumor cells to macrophage antitumor mechanisms. Photochem. Photobiol. 66: 384–388.

Reiter I, Schwamberger G and Krammer B. 1999. Activation of macrophage tumoricidal activity by photodynamic treatment in vitro – indirect activation of macrophages by photodynamically killed tumor cells. J. Photochem. Photobiol. B 50: 99–107.

Roberts WG and Berns MW. 1989. In vitro photosensitization I. Cellular uptake and subcellular localization of mono-L-aspartyl chlorin e6, chloro-aluminum sulfonated phthalocyanine, and photofrin II. Lasers Surg. Med. 9: 90–101.

Schreiber S, Gross S, Brandis A, Harmelin A, Rosenbach-Belkin V, Scherz A and Salomon Y. 2002. Local photodynamic therapy (PDT) of rat C6 glioma xenografts with Pd- bacteriopheophorbide leads to decreased metastases and increase of animal cure compared with surgery. Int. J. Cancer 99: 279–285.

Shulok JR, Wade MH and Lin CW. 1990. Subcellular localization of hematoporphyrin derivative in bladder tumor cells in culture. Photochem. Photobiol. 51: 451–457.

Simkin GO, Tao JS, Levy JG and Hunt DW. 2000. IL-10 contributes to the inhibition of contact hypersensitivity in mice treated with photodynamic therapy. J. Immunol. 164: 2457–2462.

Star WM, Marijnissen HP, Berg-Blok AE, Versteeg JA, Franken KA and Reinhold HS. 1986. Destruction of rat mammary tumor and normal tissue microcirculation by hematoporphyrin derivative photoradiation observed in vivo in sandwich observation chambers. Cancer Res. 46: 2532–2540.

Steubing RW, Yeturu S, Tuccillo A, Sun CH and Berns MW. 1991. Activation of macrophages by Photofrin II during photodynamic therapy. J. Photochem. Photobiol. B 10: 133–145.

Thibaut S, Bourre L, Hernot D, Rousset N, Lajat Y and Patrice T. 2002. Effects of BAPTA-AM, Forskolin, DSF and Z.VAD.fmk on PDT-induced apoptosis and m-THPC phototoxicity on B16 cells. Apoptosis. 7: 99–106.

Usuda J, Okunaka T, Furukawa K, Tsuchida T, Kuroiwa Y, Ohe Y, Saijo N, Nishio K, Konaka C and Kato H. 2001. Increased cytotoxic effects of photodynamic therapy in IL-6 gene transfected cells via enhanced apoptosis. Int. J. Cancer 93: 475–480.

van Hillegersberg R, Hekking-Weijma JM, Wilson JH, Edixhoven-Bosdijk A and Kort WJ. 1995. Adjuvant intraoperative photodynamic therapy diminishes the rate of local recurrence in a rat mammary tumour model. Br. J. Cancer 71: 733–737.

Vantieghem A, Xu Y, Declercq W, Vandenabeele P, Denecker G, Vandenheede JR, Merlevede W, de Witte PA and Agostinis P. 2001. Different pathways mediate cytochrome c release after photodynamic therapy with hypericin. Photochem. Photobiol. 74: 133–142.

Wilson BC, Olivo M and Singh G. 1997. Subcellular localization of Photofrin and aminolevulinic acid and photodynamic cross-resistance in vitro in radiation-induced fibrosarcoma cells sensitive or resistant to photofrin-mediated photodynamic therapy. Photochem. Photobiol. 65: 166–176.

Wyld L, Reed MW and Brown NJ. 2001. Differential cell death response to photodynamic therapy is dependent on dose and cell type. Br. J. Cancer 84: 1384–1386.

Wood SR, Holroyd JA and Brown SB. 1997. The subcellular localization of Zn(II) phthalocyanines and their redistribution on exposure to light. Photochem. Photobiol. 65: 397–402.

Yamamoto N, Homma S, Sery TW, Donoso LA and Hoober JK. 1991. Photodynamic immunopotentiation: in vitro activation of macrophages by treatment of mouse peritoneal cells with haematoporphyrin derivative and light. Eur. J. Cancer 27: 467–471.

Yamamoto N, Sery TW, Hoober JK, Willett NP and Lindsay DD. 1994. Effectiveness of photofrin II in activation of macrophages and in vitro killing of retinoblastoma cells. Photochem. Photobiol. 60: 160–164.

Ziegler K and Unanue ER. 1981. Identification of a macrophage antigen-processing event required for I-region-restricted antigen presentation to T lymphocytes. J. Immunol. 127: 1869–1875.

Ziolkowski P, Symonowicz K, Milach J, Zawirska B and Szkudlarek T. 1997. In vivo tumor necrosis factor-alpha induction following chlorin e6-photodynamic therapy in Buffalo rats. Neoplasma 44: 192–196.